Body Composition Analysis of Small Pigs by Dual-Energy X-Ray Absorptiometry[1,2]

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ABSTRACT: We evaluated the use of dual-energy x-ray absorptiometry (DXA) for measuring the gross body composition of small subjects in 97 pigs that ranged from 5 to 27 kg live body weight. Scans were performed using a Lunar DPXL densitometer in the pediatric mode (Version 3.8e). The DXA scans of the live pigs provided measurements of total fat, lean, and bone mineral content. After scanning, the pigs were killed, the entire body was ground, and samples were analyzed chemically (CHEM) for fat, protein, ash, and water content. We found that DXA significantly underestimated the percentage of fat in the body (DXA, 6.9 ± .33% vs CHEM, 10.9 ± .31%, $P < .001$). The correlation (r) between DXA and chemical measures of percentage fat was .86 and for grams of fat it was .96. Lean tissue mass measured by DXA was highly correlated with CHEM measurements of total grams of body water (r = .99), total grams of body protein (r = .94), and lean body mass (r = .99). The average DXA bone mineral content was within 2% of the amount estimated from total body ash and the correlation between the two values was .94. The relationships between DXA and CHEM measurements for percentages of body composition of pigs that weighed between 5 and 27 kg are described by the following regression equations: $\%fat_{CHEM} = 5.22 + [.817 \cdot fat_{DXA}]$, (r = .86, standard error of the estimate, SEE = 1.56); $\%protein_{CHEM} = -7.8 + [.256 \cdot \%lean_{DXA}]$, (r = .35, SEE = 2.3); $\%water_{CHEM} = -5.2 + [.808 \cdot \%lean_{DXA}]$, (r = .59, SEE = 3.67). These results are consistent with previously reported results and suggest that even though direct use of DXA readings may not be sufficiently accurate, the high degree of correlation indicates that with proper calibration the DXA values can be used to predict body composition.

Key Words: Pigs, Body Composition, X radiation, Analytical Methods, Densitometry

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Introduction

The measurement of the body composition of small pigs is useful for monitoring development during the neonatal and early weaning periods. This information may be important in evaluating the genetic, nutritional, disease, or therapeutic status of pigs. Furthermore, small pigs are frequently used as a model for human studies, including body composition assessment. Sophisticated techniques, such as x-ray computer-assisted tomography (**CAT**) (Skjervold et al., 1981; Leymaster, 1986; Luiting et al., 1995) and magnetic resonance imaging (**MRI**) (Fuller, 1985; Mitchell et al., 1991; Scholz et al., 1993; Baulain et al., 1996) have been used to measure body composition of pigs. The relative merits and applications of these accurate, but expensive techniques for measuring body composition are discussed by Vangen and Jopson (1996).

Dual-energy x-ray absorptiometry (**DXA**) shows considerable promise for measuring body composition of adult humans (Lukaski, 1987; Heymsfield et al., 1989; Mazess et al., 1990; Laskey, 1996) and larger pigs (Mitchell et al., 1996a,b; Mitchell and Scholz, 1997). Studies by Brunton et al. (1993), Ellis et al. (1994), Picaud et al. (1996), and Pintauro et al. (1996) have used small pigs in experiments designed to evaluate the use of DXA for measuring body composition in the human neonatal and pediatric range. All of the studies with small pigs indicated that, even though there was a high correlation between the DXA measurement of total body fat and the results from chemical analysis, there were substantial differences in the absolute amounts of fat

[1]Mention of a trade name does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

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Table 1. Comparison of body composition by carcass chemical analysis (CHEM) and dual-energy x-ray absorptiometry (DXA)[a]

Component	CHEM	DXA	R^2	SEE[b]	*P*
Weight, g	14,174 ± 597	13,795 ± 592	.99	227	<.001
Lean mass, g	11,930 ± 476	12,386 ± 503	.99	534	<.001
Fat mass, g	1,624 ± 99	1,071 ± 91***	.92	283	<.001
Bone mineral, g[c]	333 ± 17	338 ± 17	.81	74	<.001
		Predicted[d]			
Fat, %	10.85 ± 3.09	11.43 ± 3.35	.74	1.57	<.001
Fat, g	1624 ± 99	1707 ± 91	.92	283	<.001
Protein, %	15.35 ± .25	19.93 ± .07***	.12	2.30	<.001
Protein, g	2205 ± 100	2768 ± 110***	.89	322	<.001
Water, %	68.06 ± .46	69.38 ± .37*	.45	3.38	<.001
Water, g	9543 ± 385	9673 ± 372	.98	477	<.001

[a]Mean ± SE, n = 97.
[b]SEE, standard error of the estimate.
[c]For carcass analysis, bone mineral mass was calculated as the following: total ash – (.085·lean weight).
[d]DXA values were obtained using the following prediction equations:
Fat, % = 493.4 – [349·DXA R-value] (Mitchell and Scholz, 1995)
Protein, g = –1.062 + [.22·DXA lean] (Mitchell and Conway, 1994)
Water, g = 508 + [.74·DXA lean] (Mitchell and Scholz, 1995)
*Significantly different from carcass analysis, $P < .05$.
***Significantly different from carcass analysis, $P < .001$.

measured with the two methods. Furthermore, these differences varied with instrument manufacturer and mode of operation. The purpose of the present study was to gain a better understanding of the nature of these discrepancies using a large number of pigs over a wide range in body weights and to test the hypothesis that DXA is as accurate as chemical analysis for measuring the body composition of small pigs.

Methods

Single DXA scans were performed on a total of 97 pigs that ranged from 5 to 27 kg live body weight. Each pig was anesthetized using a mixture of ketamine, tiletamine, zolazepam, and xylazine and was then placed on the DXA table in a prone position as described previously (Mitchell et al., 1996b). Scans were performed using a Lunar DPXL densitometer in the pediatric mode (Version 3.8e). The DXA scans of the live pigs provided measurements of total fat, lean, and bone mineral content. After scanning, the pigs were euthanized with pentobarbital, and the entire body (including gut contents) was frozen and then homogenized. Samples of the homogenate were analyzed for fat (chloroform/methanol extraction with the method of Folch et al., 1957), protein (Kjeldahl N × 6.25), water (weight loss following 10 d of lyophylization), and ash (weight of residue following combustion in muffle furnace for 24 h at 520°C).

The DXA measurements of percentage fat were corrected by applying a prediction equation to the DXA R-value (Mitchell and Scholz, 1995). The R-value is the ratio of the soft tissue x-ray attenuation coefficients that DXA uses to assign the soft tissue values to fat or lean. The equation for predicting percentage fat is as follows: fat percentage = 493.4 – [349·DXA R-value], (n =73, R^2 = .90, standard error of the estimate, **SEE** = 1.72). Total body protein was estimated from DXA lean tissue mass (Mitchell and Conway, 1994) using the following equation: grams of protein = –1.062 + .22[DXA lean], (n = 30, R^2 = .93, SEE = 410 g). Water, which is the major component of the lean tissue mass, was estimated (Mitchell and Scholz, 1995) from the DXA readings using this equation: grams of water = 508 + .74[DXA lean], (n = 73, R^2 = .99, SEE = 639 g). Correspondingly, chemical analysis did not provide a measure of lean tissue mass; therefore, it was computed as the sum of total body protein and water. In order to compare DXA bone mineral content (**BMC**) to total carcass ash by combustion, the total carcass ash content was corrected for the ash content of .85% for pork meat (Jebb et al., 1995).

Comparisons using *t*-tests of means and linear regression analyses were performed using Statgraphics (1992, Version 6.0) procedures.

Results and Discussion

The results of carcass analysis and DXA measurement of body composition of small pigs are compared in Table 1. The average for the summation of the DXA tissue mass measurements was 379 g, or 2.8% (P = .65) less than the average scale measurement of the weight of the pigs at the time of scanning. This

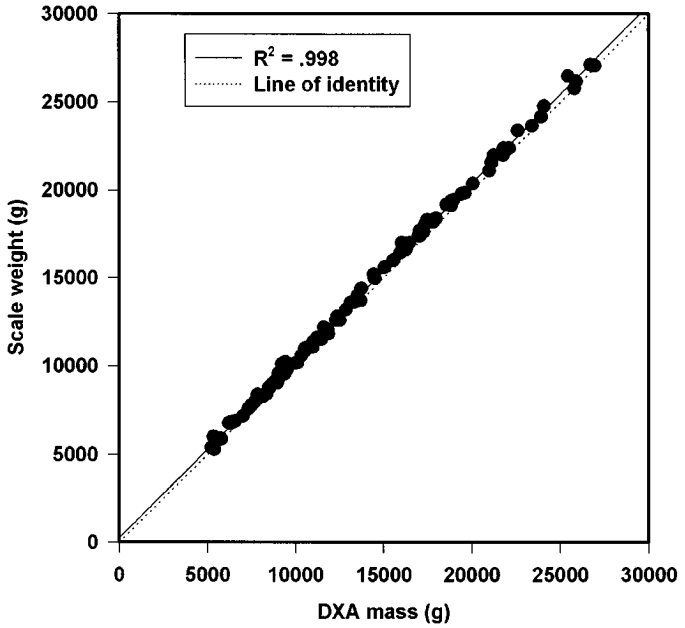

Figure 1. Relationship between dual-energy x-ray absorptiometry (DXA) mass and scale weight.

difference is similar to that observed for larger pigs (2%) using the same instrument (Mitchell et al., 1996a). The relationship between these values is illustrated in Figure 1. The high correlation between DXA mass and scale weight is consistent with the results of other studies involving small pigs (Brunton et al., 1993; Ellis et al., 1994; Picaud et al., 1996; Pintauro et al., 1996). The accurate determination of total tissue mass is a necessary aspect of the DXA methodology; however, as indicated by Roubenoff et al. (1993) and later confirmed by Mitchell et al. (1996a), this cannot be taken as evidence that DXA will accurately predict fat, lean, or bone mineral content of the body.

The average DXA value for fat mass was 553 g, or 36% less ($P < .001$) than the average fat content measured by chemical analysis. The basis for the assignment of soft tissue by DXA as either lean or fat is the DXA R-value, which is the ratio of the mass attenuation coefficients measured at the two x-ray energy levels (38 and 70 keV for the Lunar instrument). The relationship between the DXA R-values and the chemical determination of percentage fat in bodies of pigs measured in this study is shown in Figure 2. The DXA measurement of percentage of fat was consistently below that of the chemical measurement. Also, there is an obvious lack of linearity between the DXA R-values and the DXA measurements of percentage fat. This departure from linearity occurs at higher R-values (or lower percentage fat), especially at R-values greater than 1.38, for which the DXA software fails to recognize fat percentages of less than approximately 4%. The difference between DXA and chemical values also tended to be greater in pigs with less fat. This difference between DXA and chemical values was not unexpected based on a previous study (Mitchell et al., 1996a), in which concordance correlation analysis indicated that DXA would overestimate the percentage fat in pigs containing more than 20% fat and underestimate percentage fat in pigs with less than 20% fat. Based on chemical analysis, all pigs in this study had less than 20% body fat.

In general, as pigs get larger, the percentage body fat increases. Thus, the question arises, is the accuracy of DXA fat measurement more closely associated with fat content or body size? Using the relative measure of fat content (DXA fat/chemical fat·100) of each pig as an indicator of DXA accuracy, the accuracy was correlated with both body weight ($r = .65$, $P < .001$) and percentage body fat ($r = .41$, $P < .001$). However the accuracy was more highly correlated ($P < .05$) with body weight than with body fat, and, in a two-component model, body weight would account for 80% of the variance in accuracy, whereas percentage body fat accounted for only 20%. The relationship between this measure of DXA accuracy and body weight is shown in Figure 3. In the study by Brunton et al. (1993), the correlation (r) between DXA and chemical values for the fat content of pigs weighing 5.9 kg was .83, and for pigs weighing 1.6 kg it was only .06.

Because the DXA measurements of fat content were not sufficiently accurate, a prediction equation was applied, based on the relationship between the DXA R-

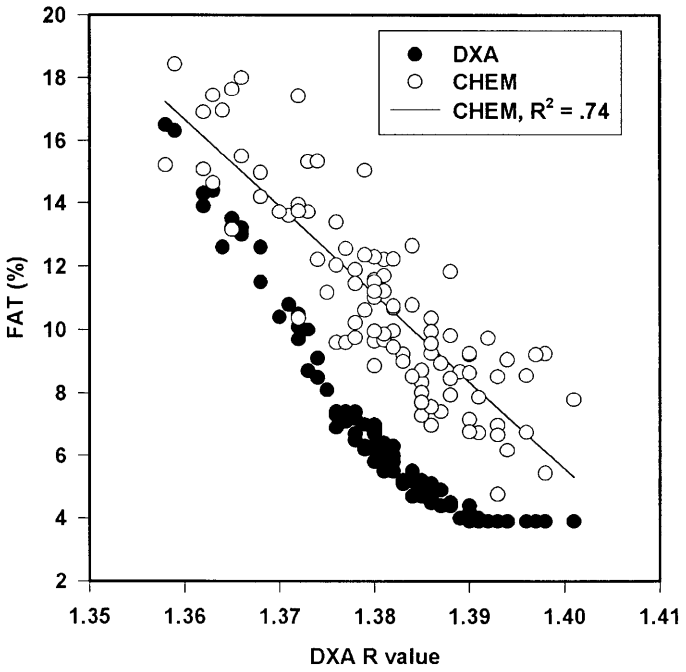

Figure 2. Relationship between dual-energy x-ray absorptiometry (DXA) R-values and percentage of fat measured by DXA or chemical (CHEM) analysis.

value and percentage fat as determined by chemical analysis. These results are included in Table 1. Because the equation that was used in Table 1 was derived primarily from larger pigs, it was revised using the data obtained from this study with only small pigs. The revised equation for predicting the percentage fat in small pigs is as follows: fat percentage = 394 – (277·DXA R-value). The relationship between the amount of fat measured by chemical analysis and the amount measured with DXA or the amount predicted from DXA R-values using the revised prediction equation is shown in Figure 4. The correlation between DXA and chemical measurements (r = .96) in the present study was similar to .989 observed previously with larger pigs (Mitchell et al., 1996a) and correlations ranging from .97 to .99 for small pigs (Ellis et al., 1994; Picaud et al., 1996; Pintauro et al., 1996).

The average DXA measurement of lean mass, consisting of all other components of the soft tissue mass excluding fat, was 456 g, or 3.8% more (P = .65) than the average chemical value of lean mass, consisting of total body protein and water (Table 1). The correlation (r) between DXA and chemical measurements of lean mass was .99, which is similar to the r = .96 to .99 reported in other studies with small pigs (Brunton et al., 1993; Ellis et al., 1994; Pintauro et al., 1996). Our earlier study with larger pigs (Mitchell et al., 1996a) reported a correlation of r = .97 and suggested that the DXA lean mass measurement could be used to predict total body protein. The relationship between DXA lean mass and total carcass protein in the present study is shown in Figure 5. The correlation (r = .96) between DXA lean mass and total body protein was similar to that observed with larger pigs. However, when the prediction equation derived from the study with larger pigs was applied to the DXA values obtained with smaller pigs it resulted in a 25% overestimation of total body protein (Table 1). This difference is also evident in Figure 5 where the prediction line is included. This indicates that a different prediction equation is needed for smaller pigs. Based on the data obtained from this study, total body protein in small pigs would be predicted by the following equation: grams of protein = (.188·DXA lean) – 157, with a SEE of 322 g.

Because water is the major component of lean mass, it is not surprising that there was a close correlation between DXA lean mass and total body water, as is shown in Figure 6. Using a prediction equation (grams of water = 508 + .74·DXA lean) based on data from another DXA study of pigs weighing between 30 and 90 kg (Mitchell and Scholz, 1995), the estimated total body water content shown in table 2 (9673 ± 372 g) was not different (P = .24) from that measured by chemical analysis (9543 ± 385 g), and the prediction line is almost identical to the regression line derived from the data obtained in the present study (Figure 6). Thus, it seems that, unlike protein, the relationship between DXA lean mass and water content may be constant over a wide range of body weights.

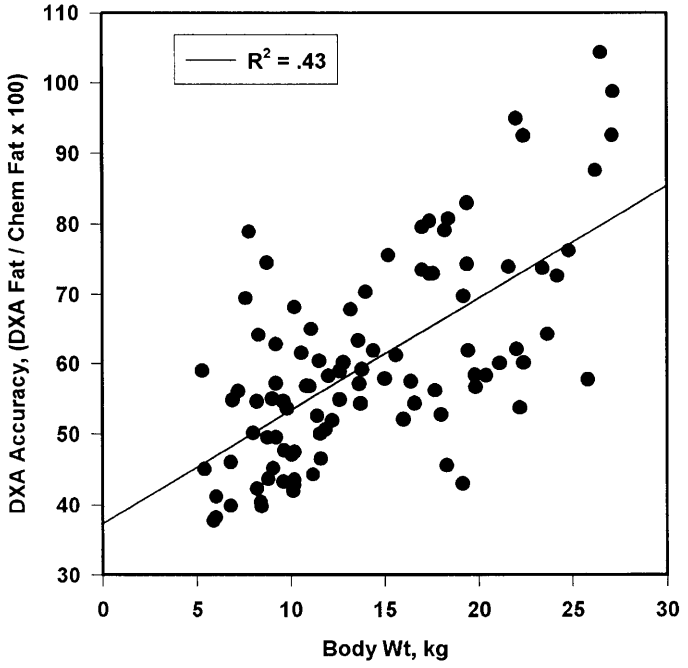

Figure 3. Influence of body weight on the relative measurement of fat by dual-energy x-ray absorptiometry (DXA) and chemical analysis (CHEM).

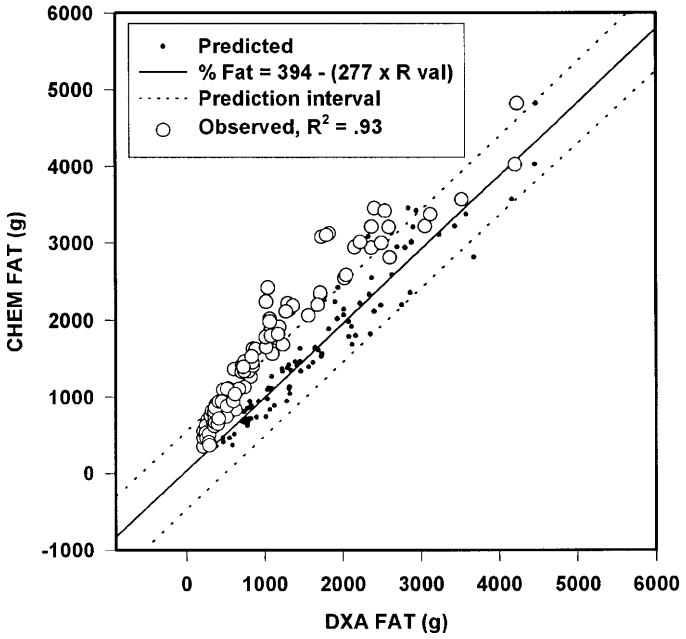

Figure 4. Relationship between dual-energy x-ray absorptiometry (DXA) fat mass and total body fat by chemical (CHEM) analysis. Prediction interval was generated using Sigma Plot for Windows, Version 2.01 (1994).

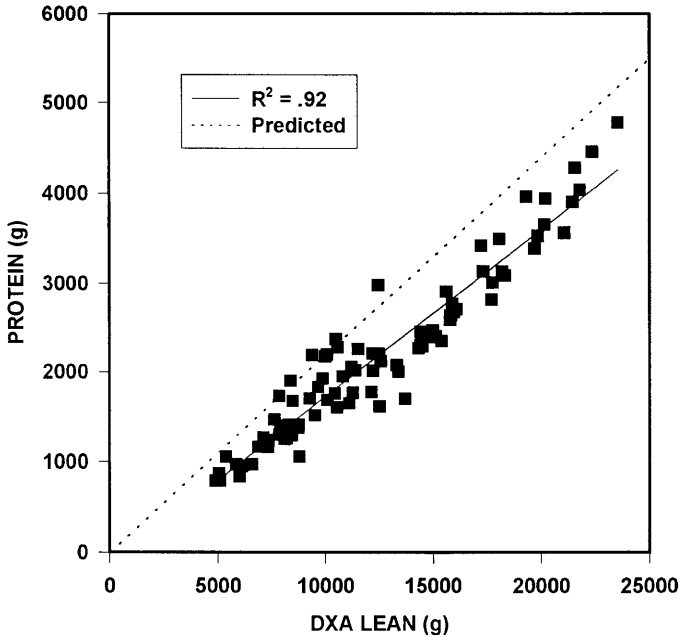

Figure 5. Relationship between dual-energy x-ray absorptiometry (DXA) lean mass and total carcass protein by chemical analysis or protein predicted using the following equation: protein (g) = –1.062 + [.22·DXA lean] (Mitchell and Conway, 1994).

As shown in Table 1, the average bone mineral content (BMC) measured with DXA was only 1.4% more (P = .19) than that estimated from total body ash (total body ash less soft tissue ash content). By comparison, DXA BMC was 3.5% less than the estimate from body ash in 90-kg pigs (Mitchell et al., 1996b). In addition to the close agreement for the quantity of bone mineral being measured, there was also a close correlation between the BMC and ash values (Figure 7). In the other studies involving small pigs, Picaud et al. (1996), Ellis et al. (1994), and Pintauro et al. (1996) reported DXA BMC values of 48, 73, and 90% of total body ash content, respectively, with correlations between the two values of .96, .99, and .97, respectively. Brunton et al. (1993) found DXA BMC to be 70% of total body ash for pigs that weighed 1.5 kg, but 99% for pigs that weighed 5.9 kg. In the latter study, the weight ranges were small, which resulted in low correlations.

In general, the results of this study are in agreement with the previous studies in which DXA was evaluated using small pigs. The main problem seems to be a lack of accuracy in measuring body fat content. Even though variability from one study to the next regarding the measurement of fat is in part due to differences among DXA instrument manufacturers and the version of software being used, the results of the present study suggest that weight of the pigs may be a major factor. Early investigations regarding the use of DXA for measuring body composition indicated that the DXA measurement was independent of tissue depth (Mazess et al., 1990). However, it should be recognized that there are limits, and the lower limit is probably on the order of 1 to 2 cm. On average, even the smallest of pigs would be well above this limit; however, the smaller the pig, the greater the proportion of tissue or pixels (along the edges of the scan) that would fall below this limit. This could result in a higher noise-to-signal ratio. One obvious conclusion of the present study is the failure of the software to recognize a body fat content of less than approximately 4%. At birth and under conditions of malnourishment, the body fat content of pigs could easily be in the range of 1.5 to 3%.

In studies with hemodialysis patients, an increase in the hydration status (with no apparent increase in total body protein) increases the DXA measurement of lean mass (Horber et al., 1992). Dramatic changes in tissue hydration during early development may seriously influence the interpretation of DXA measurement of soft tissue composition. McMeekan (1940) reported that the water content of subcutaneous adipose tissue of pigs was 84.9% at birth, dropping to 19.5% by 4 wk of age and to 4.9% at 28 wk, whereas the lipid content increased from 6.2% at birth to 75.4% at 4 wk and to 92.4% at 28 wk. Kauffman et al. (1964) observed that the protein-to-water ratio in pork muscle increased rapidly from .156 at birth to .297 at 3.5 mo of age. Assuming that the attenuation of the x-ray beam by lipid vs. water is not affected by

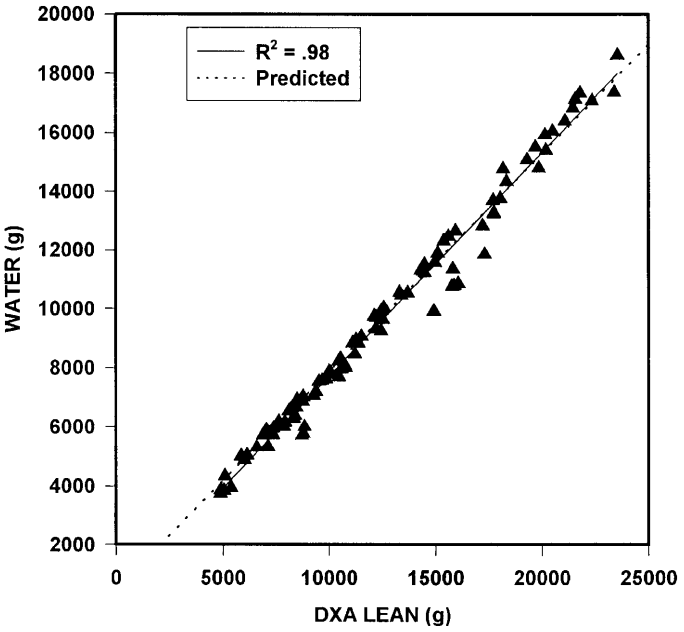

Figure 6. Relationship between dual-energy x-ray absorptiometry (DXA) lean mass and total body water by chemical analysis or water predicted using the following equation: water (g) = 508 + [.74·DXA lean] (Mitchell and Scholz, 1995).

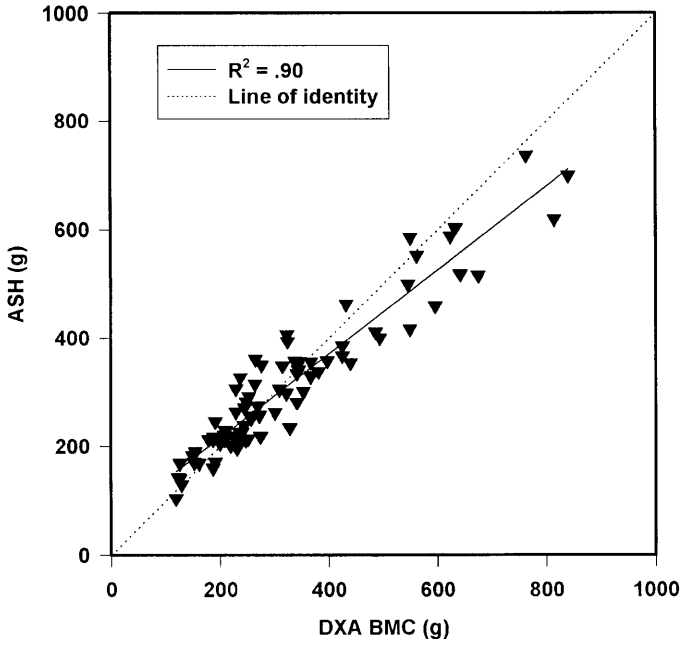

Figure 7. Relationship between dual-energy x-ray absorptiometry (DXA) bone mineral content (BMC) and carcass ash.

how these two components are disbursed throughout the tissue, tissue hydration would be expected to have little effect on the DXA assessment of fat content. On the other hand, changes in the hydration of both adipose and muscle tissue would have significant influence on how the DXA lean mass measurement is allocated between water and protein. When the same equation as used here to predict protein from DXA lean was used in an earlier study with 90-kg pigs, it gave a slightly greater value for protein than was measured by chemical analysis (Mitchell et al., 1996b). In the study with 90-kg pigs, the protein-to-water ratio by chemical analysis was .285 compared to a mean value of .225 for the small pigs used in this study. This would be a likely explanation for why the protein content of pigs in this study was 25% less than that predicted using an equation based on data from larger (older) pigs. Conversely, the increased hydration in young pigs could result in an underestimation of water based on the DXA lean measurement. However, with water being a much larger component of the lean mass, the relative impact on estimation of total body water would be considerably less than for total body protein.

Implications

The results of this study are consistent with previous reports of difficulties in obtaining a reliable measurement of the fat content of small pigs using dual-energy x-ray absorptiometry (DXA). The present results indicate that the problem becomes more severe with decreasing body weight. However, the correlations between DXA and chemical measurements are high enough to suggest that, with proper calibration based on chemical analysis, the DXA measurement could provide a valid measure of body fat in small pigs and that, using tissue hydration factors based on age or maturity, water and protein content could also be predicted. Thus, we recommend that these calibrations be specific with regard to weight range, make of DXA instrument, and version of software.

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